Central Vasotocin-Immunoreactive System in a Male Passerine Bird (*Junco hyemalis*)

G.C. PANZICA,[1]* L. PLUMARI,[1] E. GARCÍA-OJEDA,[1] AND P. DEVICHE[2]
[1]Department of Anatomy, Pharmacology, and Forensic Medicine, University of Torino, I-10126 Torino, Italy
[2]Institute of Arctic Biology, University of Alaska at Fairbanks, Fairbanks, Alaska 99775-7000

ABSTRACT

Previous investigations have identified regions of the avian brain that contain immunoreactive vasotocinergic (VT-ir) cell bodies and fibers. These studies exclusively used domesticated species, and the relevance of the findings for free-living birds has not been established. The present study used immunocytochemistry to determine the neuroanatomical distribution of the VT-ir system in the brain of a well-studied male passerine bird (dark-eyed junco, *Junco hyemalis*) obtained from a natural population in interior Alaska (65°N, 147°W). VT-ir cell bodies were observed in several brain regions (paraventricular and supraoptic nuclei, nucleus of the stria terminalis), where they have been described in other oscine species. VT-ir fibers were widespread in many brain regions and were especially abundant in the medial preoptic nucleus, the basal region of the septum, and the hypothalamic–neurohypophyseal tract. Fibers were also present in brain regions that are involved in the control of vocal behavior including the ventromedial capsular region of the nucleus robustus archistriatalis and the dorsomedial portion of the mesencephalic nucleus intercollicularis. The widespread brain distribution of VT-ir cell bodies and fibers in juncos generally resembles that of domestic birds and suggests a role for this neuropeptide in the control of reproductive behavior and physiology. J. Comp. Neurol. 409:105–117, 1999.

Indexing terms: songbirds; hypothalamus; diencephalon; vasopressin; nucleus of the stria terminalis; preoptic area

Vasotocin (VT) is a nonapeptide produced in the hypothalamohypophysial system of nonmammalian vertebrates and is closely related to mammalian vasopressin (VP; for a recent review on the evolutionary aspect of this family of neuropeptides, see Acher et al., 1993; Acher and Chauvet, 1995). VT and VP were originally identified as neurohypophysial hormones produced by hypothalamic magnocellular elements and regulating hydromineral balance (Morley and Silver, 1992; Leng et al., 1992). Due to the role played by these neuropeptides in osmoregulation, they are also called antidiuretic hormones (ADH). VT and VP are also secreted at the level of the median eminence and probably by numerous nerve terminals within several brain regions, where they regulate the adenohypophyseal production of hormones (Makara et al., 1996) and various brain functions and behaviors (Dantzer and Bluthé, 1993; Insel et al., 1993).

In birds, the physiological functions classically attributed to VT are the regulation of water and electrolytic balance (for reviews, see Simon Oppermann et al., 1988; Ramieri and Panzica, 1989) and of blood pressure (Szczepanska-Sadowska et al., 1985). Like VP in mammals, VT in birds is involved in the control of reproduction including oviposition (Rzasa and Ewy, 1982; Rice et al., 1985; Shimada et al., 1986; Koike et al., 1988), male sexual behavior (Kihlström and Danninge, 1972; Bernroider and Leutgeb, 1994; Leutgeb, 1995; Castagna et al., 1998; Goodson and Adkins-Regan, 1999) and aggressive behav-

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E. García-Ojeda's current address: Department of Cell Biology and Pathology, University of Salamanca, Salamanca, Spain.

*Correspondence to: Dr. GianCarlo Panzica, Department of Anatomy, Pharmacology, and Forensic Medicine, Laboratory of Neuroendocrinology, c.so M. D'Azeglio 52, I-10126 Torino, Italy. E-mail: giancarlo.panzica@unito.it

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ior (Goodson, 1998a,b; Goodson and Adkins-Regan, 1999), and vocalizations (Voorhuis et al., 1991a; Castagna et al., 1998).

The localization of VT and/or its carrier protein (neurophysin) in the hypothalamoneurohypophysial system of several avian species has been determined with immunocytochemical techniques (for full review of the literature, see Jurkevich et al., 1996; Panzica et al., 1997). This localization has been confirmed by in situ hybridization studies in galliforms (Chaturvedi et al., 1994; Aste et al., 1996, 1998; Jurkevich et al., 1997). The results of these investigations suggest that the distribution of magnocellular neurosecretory elements is similar across nonoscine avian species and comparable to that of mammals (Sánchez et al., 1991; Viglietti-Panzica and Panzica, 1991).

It is normally very difficult to match the localization of immunocytochemically detectable peptidergic cell groups and cerebral nuclei with that of brain regions defined by classic neuroanatomical methods such as the Nissl stain. This difficulty explains the use of different terminologies by different investigators to describe the anatomical distribution of peptidergic systems, and this confusion could result in inaccurate homologies with other vertebrates (for further discussions, see Fasolo et al., 1988; Sánchez et al., 1991; Viglietti-Panzica and Panzica, 1991; Panzica et al., 1997; Moore and Lowry, 1998). For this reason, Berk et al. (1982) suggested a nomenclature for magnocellular VT cell groups in the pigeon brain based exclusively on the topographic position of VT-immunoreactive (VT-ir) cell clusters identifying periventricular, lateral, and dorsal diencephalic groups. This nomenclature was subsequently used for the study of other avian species (Japanese quail, domestic mallard, domestic fowl) and has been proposed as a general rule for nonoscine birds (Viglietti-Panzica, 1986).

The application of ICC methods and of in situ hybridization techniques has led to the discovery of a broad distribution of VT-ir cell bodies (smaller and less intensely labeled than those in the so-called magnocellular neurons) and fibers that are not directly connected to the hypothalamoneurohypophysial system. Scattered or clustered VT-ir neurons have been observed in the lateral diencephalic region (Panzica et al., 1988; Aste et al., 1996), whereas a rather large group of parvocellular VT-ir elements has been identified in the avian nucleus of the stria terminalis, pars medialis (BSTm), in both oscine birds (Kiss et al., 1987; Voorhuis et al., 1988b; Voorhuis and De Kloet, 1992) and galliforms (Aste et al., 1996, 1998; Jurkevich et al., 1996, 1997).

Previous studies on the distribution of avian VT-ir cell bodies and fibers were performed with exclusively domesticated species. To investigate whether results obtained with these species extend to free-living birds, we used ICC to localize VT-ir cell bodies and fibers in the brain of a nearctic oscine bird, the dark-eyed junco (*Junco hyemalis*), obtained from a wild population during the breeding season. Dark-eyed juncos commonly breed in many regions of North America. They are sexually dimorphic and photoperiodic and have been used extensively as an experimental model for eco- and neurophysiological investigations (Deviche and Güntürkun, 1992; Saldanha et al., 1994; Gulledge and Deviche, 1995, 1997; Ketterson et al., 1996).

MATERIALS AND METHODS

Animals

Five male dark-eyed juncos were collected from a local population in Fairbanks, Alaska (65°N, 148°W) on 10 May 1995 (natural photoperiod: 17.5 hours light:6.5 hours dark) and killed within 7 hours of capture. At the time of capture, cloacal protuberance (an androgen-dependent secondary sexual characteristic; Schwabl and Farner, 1989; Deviche, 1992, 1995) widths were measured to the nearest 0.1 mm with calipers. After death, testes were excised and placed in buffer until weighing to the nearest milligram the same day. Birds were caught under necessary state and federal scientific bird collecting permits, and all experimental procedures were approved by the University

Abbreviations

A	archistriatum
AQ	aqueductus cerebri
AVT	area ventralis (Tsai)
BSTl	nucleus of the stria terminalis, pars lateralis
BSTm	nucleus of the stria terminalis, pars medialis
CA	commissura anterior
Cb	cerebellum
CO	chiasma opticum
CPa	commissura Pallii
DSD	decussatio supraoptica dorsalis
DSV	decussatio supraoptica ventralis
E	ectostriatum
EW	nucleus of Edinger-Westphal
FLM	fasciculus longitudinalis medialis
FPL	fasciculus prosencephalicus lateralis
FRL	formatio reticularis lateralis mesencephali
FRM	formatio reticularis medialis mesencephali
GCT	substantia grisea centralis
GLV	nucleus genicolatus lateralis, pars ventralis
HV	hyperstriatum ventrale
ICo	nucleus intercollicularis
LA	nucleus lateralis anterior thalamis
LPO	lobus parolfactorius
LoC	locus coeruleus
MLd	nucleus mesencephalicus lateralis, pars dorsalis
MLv	nucleus mesencephalicus lateralis, pars ventralis
MNV	nucleus mesencephalicus nervi trigemini
N	neostriatum
NC	neostriatum caudale
nCPa	nucleus of the commissura Pallii
nIV	nucleus nervi trocleari
NIII	nervus oculomotorius
NIV	nervus trochlearis
OMd	nucleus nervi oculomotorii, pars dorsalis
OMv	nucleus nervi oculomotorii, pars ventralis
PA	paleostriatum augmentatum
POA	anterior preoptic area
POM	nucleus preopticus medialis
PVN	nucleus paraventricularis
RA	nucleus robustus archistriatalis
Rt	nucleus rotundus
S	nucleus septalis
SCN	nucleus suprachiasmaticus
SGC	stratum griseum centrale
SL	nucleus septalis lateralis
SM	nucleus septalis medialis
SON	nucleus supraopticus
TeO	tectum opticum
Tn	nucleus teniae
TIO	tractus isthmo-opticus
TrO	tractus opticus
TrSM	tractus septomesencephalicus
Tu	nucleus tuberis
V	ventricle
VMN	ventromedial nucleus

of Alaska Fairbanks Institutional Animal Use and Care Committee.

Perfusion

Birds were deeply anesthetized with an intramuscular injection of xylacine-ketamine solution. They then received an intracardiac injection of heparin (0.3 ml; 1,000 IU/ml in 0.1 M phosphate buffer solution, pH 7.2–7.4; PB), followed by PB (20 ml) at room temperature and 4% paraformaldehyde solution in PB (25 ml). Brains were postfixed in situ in 4% paraformaldehyde overnight, dissected from skulls, transferred to PB containing 0.1% Na azide, and stored at 4°C until further processed.

Immunocytochemistry

Brains were placed in 30% sucrose in PB at 4°C for at least 48 hours. They were then frozen in mounting medium and kept at −80°C until sectioning. Thirty-micrometer-thick coronal sections were collected in sequence and kept in separate vials in a cryoprotectant solution (Watson et al., 1986) at −20°C. One section every 90 μm was stained with toluidine blue. Adjacent sections were immunostained for VT after an overnight wash in 0.01 M phosphate buffered saline (PBS).

To block endogenous peroxidase activity, sections were immersed in a solution of methanol/hydrogen peroxide (Streefkerk, 1972) for 30 minutes. Floating sections were incubated overnight at room temperature with anti-VP serum (a generous gift of M. Sofroniev, Oxford, UK) at a dilution of 1:6,000 in PBS, pH 7.3–7.4, containing 0.2% Triton X-100. A biotinylated anti-rabbit serum (Vector Laboratories, Burlingame, CA) was then used at a dilution of 1:200. The antigen–antibody reaction was revealed by a biotin-avidin system (Vectastain Elite Kit, Lab-Tek, Naperville, IL). Peroxidase activity was visualized with a solution containing 0.15 mg/ml 3,3-diaminobenzidine (DAB) and 0.025% hydrogen peroxide in 0.05 M Tris-HCl buffer, pH 7.6. Sections were mounted on chromalum-coated slides, air dried, washed in xylene, and coverslipped.

Antibody specificity

The anti-VT serum employed in this research was originally developed by Sofroniew (Sofroniew et al., 1978; Sofroniew and Weindl, 1978) in rabbits treated with arginine-vasopressin. The specificity of the antibody was tested by radioimmunoassay against VP, VT, oxytocin, mesotocin, and angiotensin II. The specificity of the antibody for use in immunocytochemistry was tested by adding VT or mesotocin (Bachem, Bubendorf, Switzerland; 200 μg antigen/ml antiserum at working dilution) to the primary antiserum before the immunohistochemical procedure. The antiserum preabsorbed with VT did not stain either cell bodies or intra- or extrahypothalamic fibers. In contrast, no detectable loss of immunoreactivity was observed after preabsorption with mesotocin.

Data analysis

Sections were observed on a Zeiss Axioplan I microscope and photographed with the use of a Kodak Wratten gelatin filter (n. 44 or 75) to increase the contrast of DAB reaction products. The nomenclature adopted in this study is largely based on the canary stereotaxic atlas (Stokes et al., 1974), with some modifications according to a recent study on the zebra finch (Balthazart et al., 1996) and to the study by Aste et al. (1998) for the definition of the nucleus of the stria terminalis.

RESULTS

Reproductive status

At the time of capture and death, juncos had partly developed testes (mean ± S.D. = 172 ± 94 mg) and cloacal protuberances (inner diameter = 4.4 ± 0.5 mm), confirming that they were photostimulated.

Overall VT-ir distribution

VT-ir neuronal cell bodies are located in three diencephalic regions: (1) a periventricular hypothalamic region that extends medially on both sides of the third ventricle, from the preoptic region, rostrally, to the dorsal anterior hypothalamus, caudally; (2) a lateral diencephalic region that originates in the preoptic area and continues in the lateral hypothalamus; and (3) a dorsal diencephalic region including the dorsal hypothalamus, the dorsal thalamus, and the septal region of the telencephalon.

In addition, VT-ir cell bodies are observed in the limbic zone of the telencephalon. VT-ir fibers are widespread in many brain areas including the brainstem. The detailed distribution of ir cells and fibers is summarized in a series of drawings through the rostrocaudal extent of the brain (Figs. 1–3).

Distribution of VT-ir cells

Periventricular region. A large cluster of cells is located in the region surrounding or close to the ventricular wall of the preoptic recess (anteriorly) and of the third ventricle (more caudally; Fig. 1A–F). Neurons in this region have markedly different sizes, shapes, and dendritic arborization. Rostrally, the first cluster of ir neurons appears at the level of the anterior preoptic area (POA), where cells, frequently aligned in chains, seem to line up the entire wall of the preoptic recess; they belong to the magnocellular type and are heavily stained (Figs. 1A–B, 4A). VT-ir cell bodies are located very close to the ependymal wall, and the cell body sometimes seems to be placed among the ependymal elements, which is a common feature of the periventricular region elements (see Fig. 4D). VT-ir elements possessing thick dendritic processes whose orientation is parallel or perpendicular to the walls of the third ventricle are frequently found in this region. More caudally, the number of large ir cell bodies very close to the ependymal layer is reduced. These cell bodies are clustered in a more limited, laterally located portion of the third ventricle wall (Figs. 1C–F, 2A–C, 4B–F) and they delineate a well-recognizable cluster that we have named paraventricular nucleus (PVN). They are usually heavily labeled and are intermingled with lightly stained perikarya (Fig. 4C,E,F). The neurons of this region are medium to large, diffusely organized, and generally multipolar. The cluster of neurons delineating the PVN extends to the level of the anterior commissure (CA). A comparison with toluidine blue–stained adjacent sections shows that the so-called paraventricular magnocellular nucleus described in the canary atlas (Stokes et al., 1974) is located immediately dorsal to the PVN identified by means of immunocytochemistry. The location of this cluster in Nissl-stained neurons coincides with that of the medial preoptic nucleus (POM), which was initially defined in the Japanese quail (for a

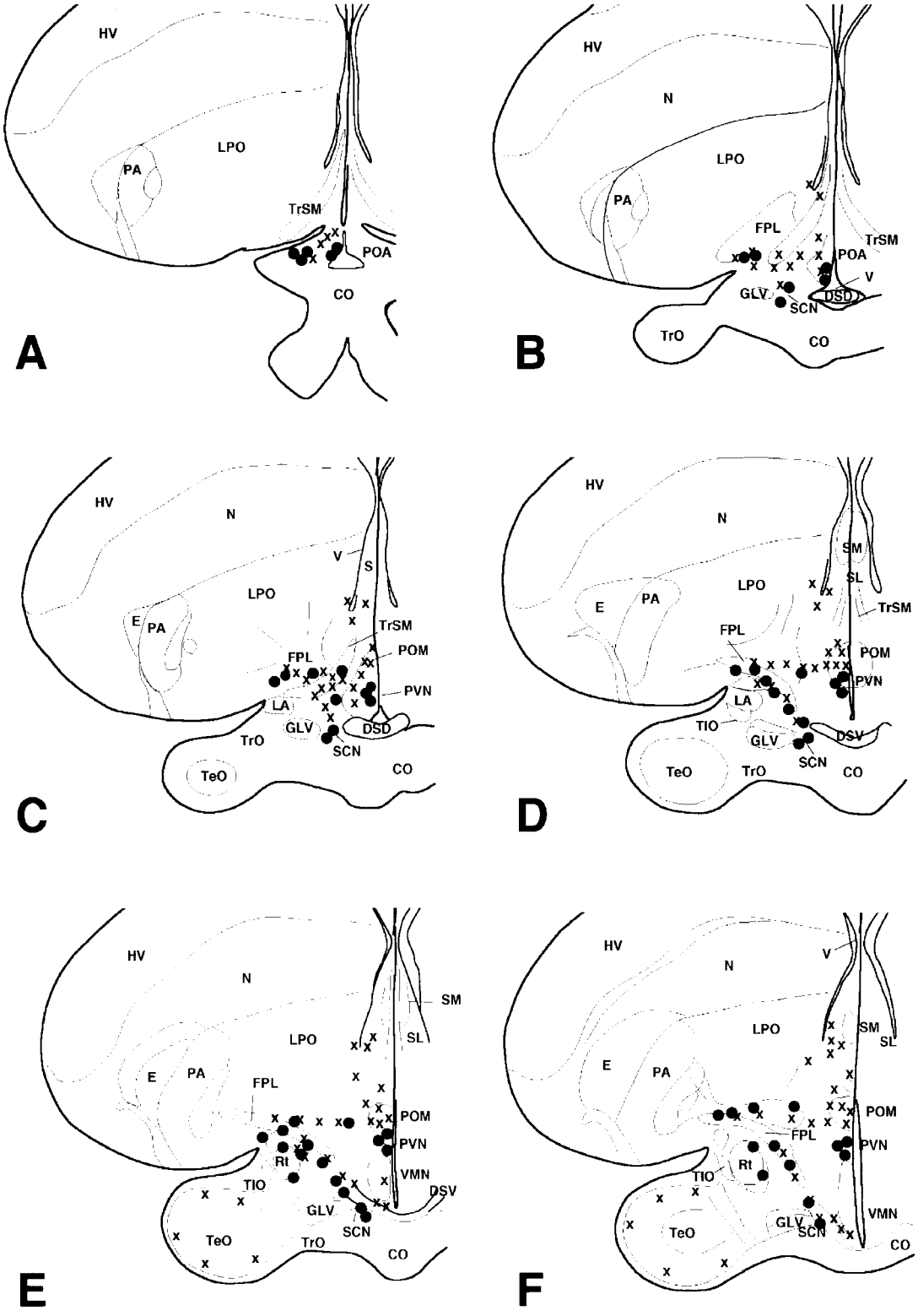

Fig. 1. **A–F:** Schematic representation of coronal sections through the dark-eyed junco rostral forebrain. The figures show the distribution of vasotocinergic-immunopositive cell bodies (black dots) and fibers and/or varicosities (x) in sections arranged from the beginning of preoptic region (A) to a level immediately preceeding the anterior commissure (F). For abbreviations, see list.

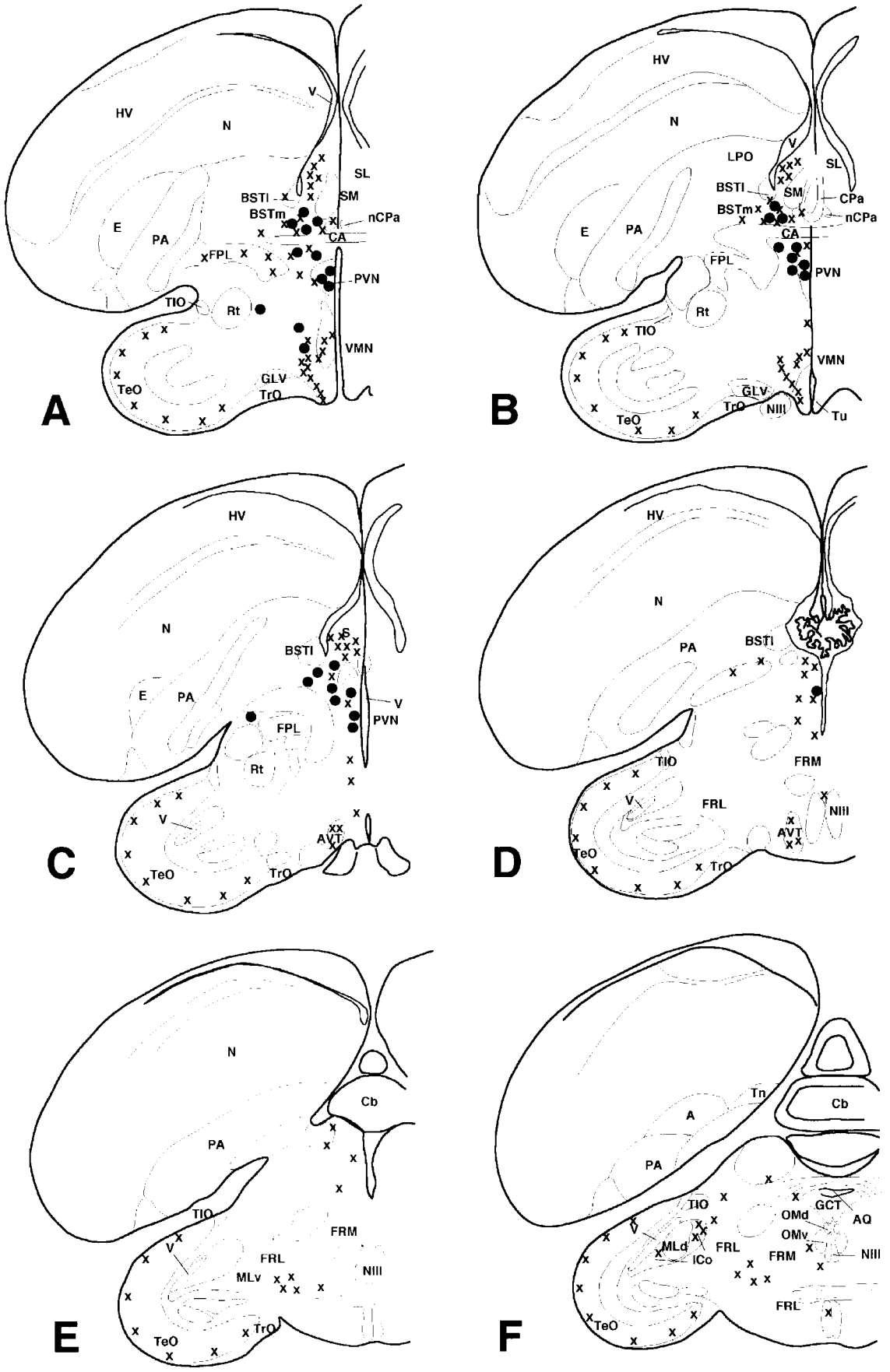

Fig. 2. **A–F:** Schematic representation of the distribution of vasotocinergic-immunoreactive cells and fibers in coronal sections through the dark-eyed junco caudal forebrain and mesencephalon. The figures show sections arranged from the level of the anterior commissure (A) to a level corresponding to the oculomotor nerve nucleus (F). For abbreviations, see list.

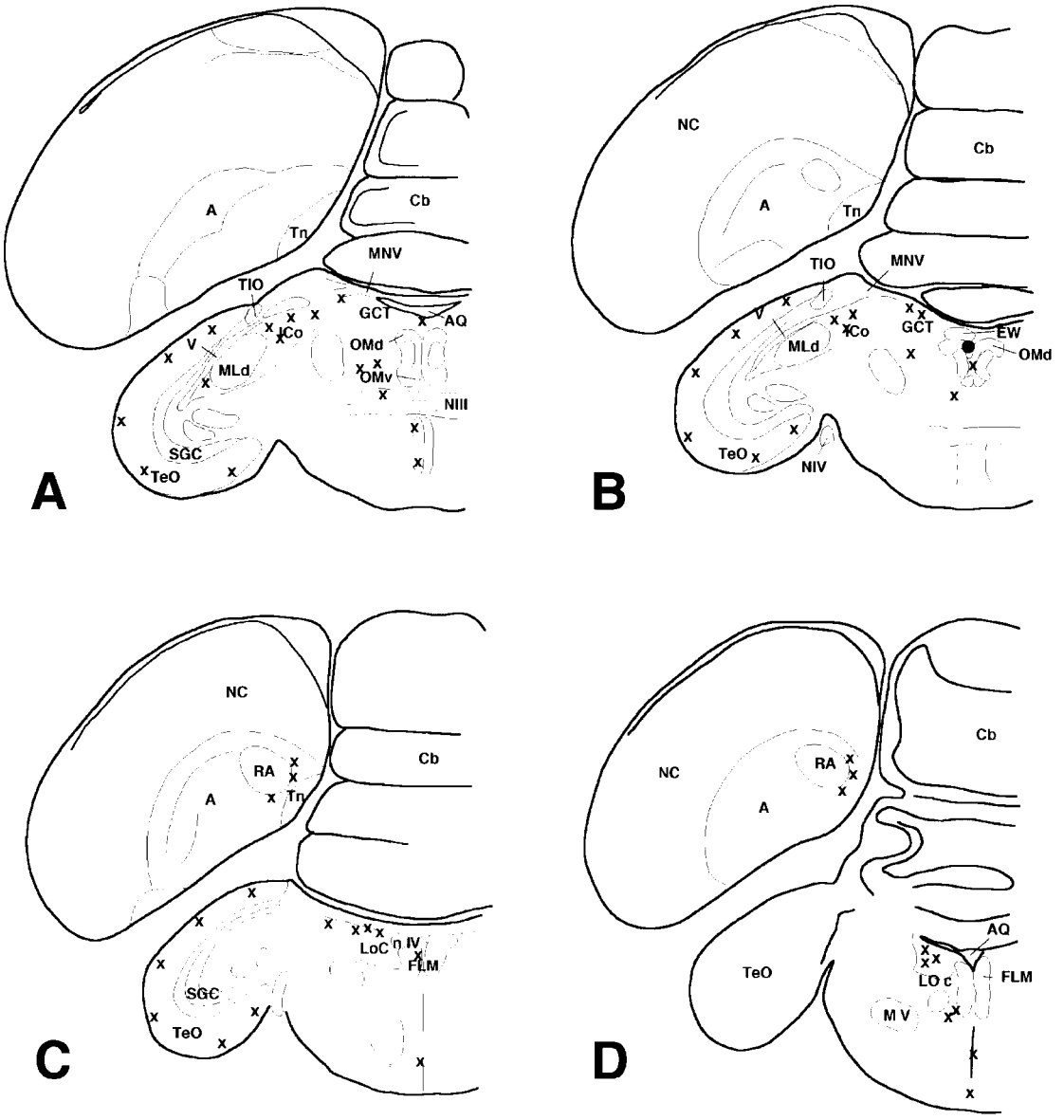

Fig. 3. **A–D:** Schematic representation of the distribution of vasotocinergic-immunoreactive cells and fibers in coronal sections through the dark-eyed junco caudal forebrain, mesencephalon, and pons. The figures show sections arranged from the level of the oculomotor nerve nucleus (A) to a level corresponding to the locus coeruleus (D). For abbreviations, see list.

complete list of references, see Panzica et al., 1996), recently described in zebra finches (Balthazart et al., 1996), and confirmed by our preliminary study in junco by means of aromatase immunocytochemistry (Plumari et al., 1998).

Lateral region. A large cluster of immunoreactive cells extends into the lateral POA and the lateral hypothalamus, and several elements are intermingled within the fibers of the lateral forebrain bundle (FPL). The main cluster of medium to large elements starts from the POA as a conspicuous and densely packed group of heavily stained neurons (Fig. 1A–C). More caudally, VT-ir cell bodies become more diffusely organized and are displaced dorsolaterally (Fig. 5A). They can be divided into a dorsolateral component, located dorsally to the FPL, and a ventromedial component, located laterally to the lateral anterior nucleus thalami (LA). This group of medium-sized and moderately stained cells correspond to the supraoptic nucleus (SON) and persists along the rostrocaudal extension of the hypothalamus (Figs. 1D–F, 5B). A separate

Fig. 4. Vasotocinergic (VT)-immunoreactive cells in the periventricular hypothalamic region. **A:** Magnocellular perikarya in the anterior preoptic area, in proximity to the borders of the preoptic recess (star). **B:** Periventricular neurons clustered in the correspondence of the rostral part of the nucleus paraventricularis (PVN). **C,E,F:** At the same enlargement, subsequent levels of the PVN; parvocellular and magnocellular VT elements are present; the magnocellular perikarya seem to be immersed in the thickness of the ependymal layer (*, third ventricle). **D:** Morphology of some isolated elements in the periventricular region: there are perikarya with thick dendritic processes parallel to the walls of the third ventricle and cell bodies with a dendrite perpendicular to the walls of the ventricle (arrow). Scale bars = 100 μm.

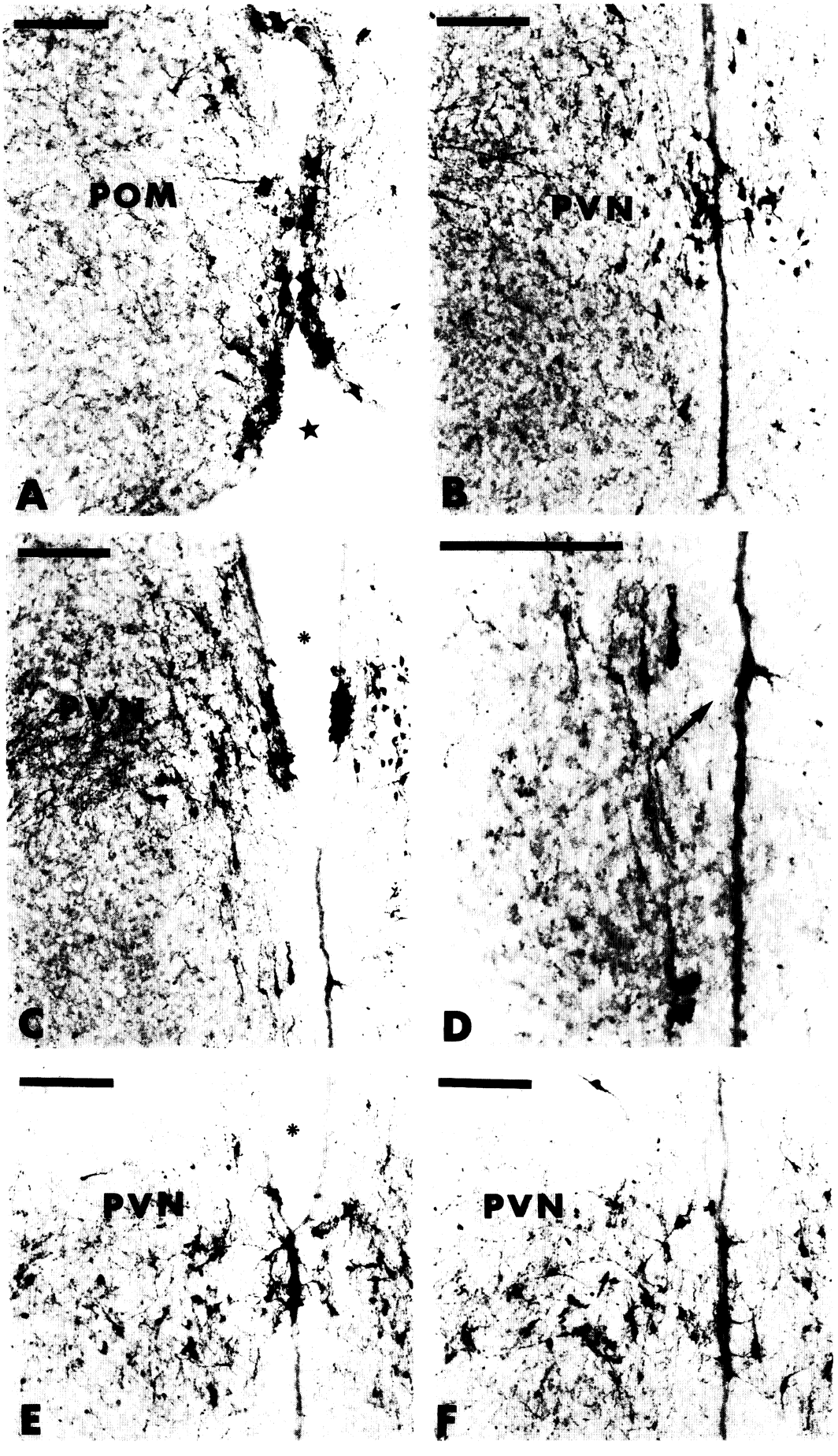

Figure 4

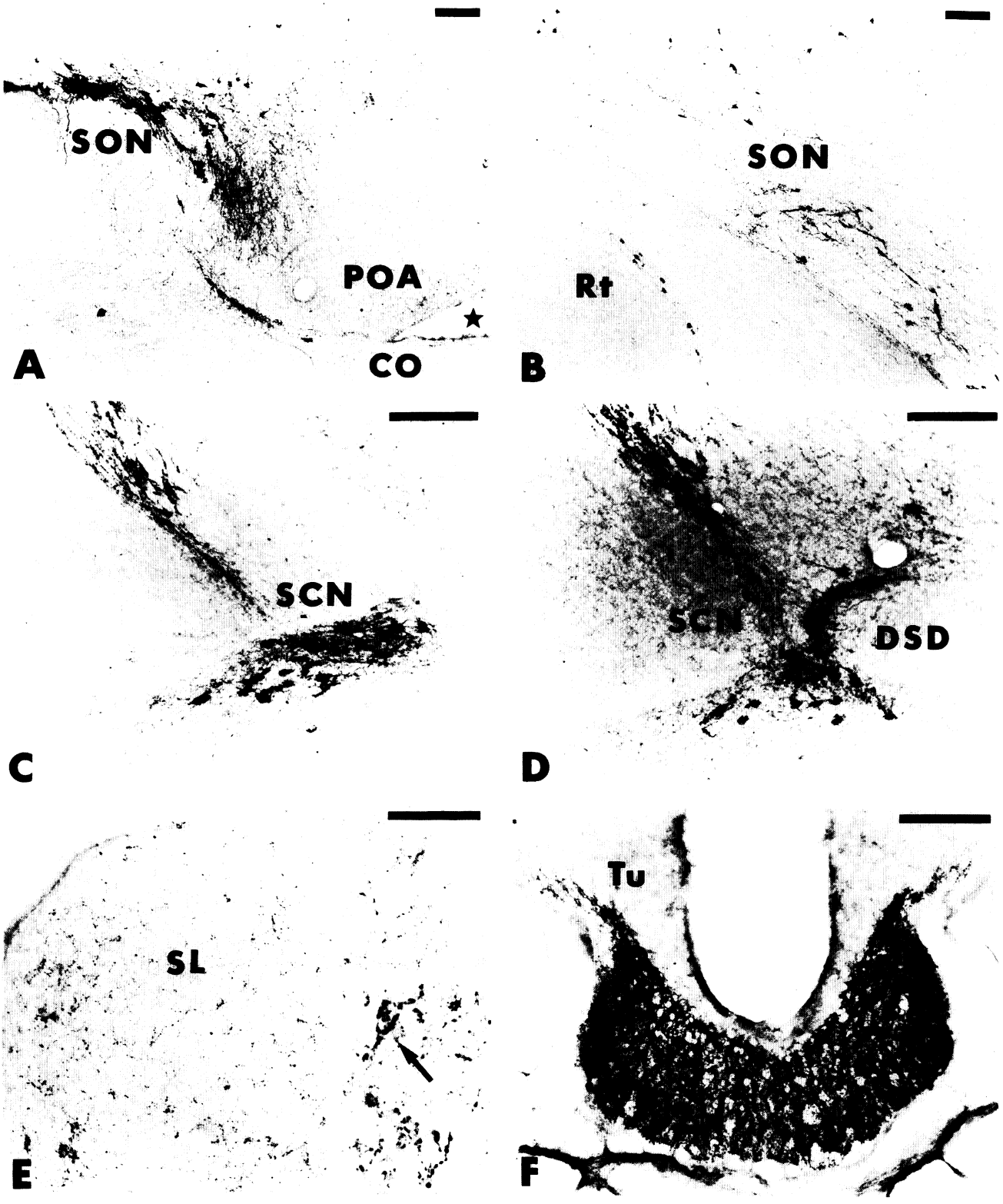

Fig. 5. **A,B:** Vasotocinergic (VT)-containing neurons in the nucleus supraopticus (SON). Rostrally (A), they are densely clustered; caudally (B), they appear more diffuse; note the few stained neurons on the dorsomedial border of the nucleus rotundus (Rt). **C,D:** Immunoreactive cells in the nucleus suprachiasmaticus (SCN) at two rostrocaudal levels; note the juxtaposition of VT neurons to the decussatio supraoptica dorsalis (DSD); the fibers of hypothalamohypophysarium bundle are visible. **E:** Vasotocinergic innervation in the septal region; the insert shows the punctate fibers that surround negative cells. **F:** The median eminence of the neurohypophysis is heavily stained. For abbreviations, see list. Scale bars = 100 μm in A–F, 50 μm for the insert in E.

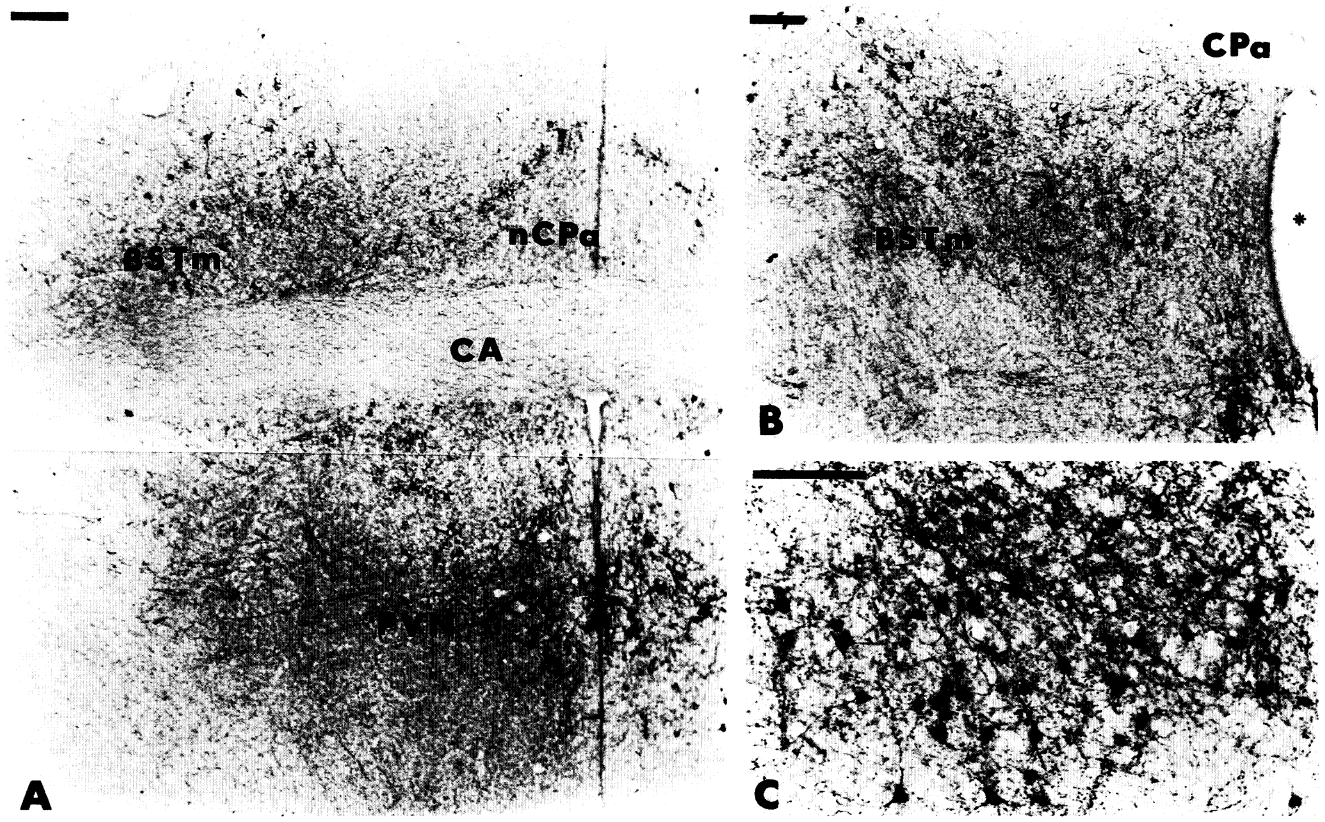

Fig. 6. Dorsal region of the vasotocinergic system. **A:** Composite. **B:** Immunoreactive medium-sized neurons in the dorsal hypothalamus and in the nucleus of the stria terminalis (BST) at two different levels (A, rostral; B, caudal). **C:** Magnified view of the vasotocinergic cells of the BST in a densely innervated zone. For abbreviations, see list. Scale bars = 100 μm.

group of VT-ir elements covers the dorsal border of the chiasma opticum (CO), medially to the ventral part of the nucleus geniculatus lateralis (GLV), and laterally to the decussatio supraoptica ventralis (DSV). The location of this cluster (Fig. 1C–F) corresponds to the nucleus suprachiasmaticus (SCN), as defined by Cassone and Moore (1987) in the house sparrow. It persists in this position until the end of the DSV, at the level of the hypothalamic tuber (Tu). SCN neurons are medium to large, and they are less intensely labeled than the magnocellular type found in the periventricular region (Fig. 5C–D).

Dorsal region. This region includes a population of VT-ir neurons that extends from the dorsal diencephalon to the limbic region. This cluster starts dorsally to the PVN at the level of the CA and extends in dorsolateral direction in a region corresponding to the nucleus of the stria terminalis, pars medialis (BSTm; Fig. 2A–C). In this region, VT-ir neurons are rather abundant and rounded. They are medium to small and moderately stained (Fig. 6A–C).

Distribution of VT-ir fibers

The junco brain contains two types of VT-ir fibers. Thick, varicose fibers leave the PVN and the SON and run into the lateral hypothalamus to turn ventrally in the direction of the median eminence and of the neurohypophysis. These fibers belong to the hypothalamoneurohypophyseal tract and end in the median eminence (external layers) and in the neurohypophysis. In the median eminence, immunoreactive fibers and varicosities are found in the internal and in the palisadic external layer (Fig. 5F).

Thinner VT-ir fibers are observed in several regions, including the telencephalon, diencephalon, midbrain, and pons. The morphology of these fibers varies, from short or longer threads to punctate structures, suggesting the existence of terminal fields. In the telencephalon, a pronounced innervation is found in the limbic region, in particular in the BSTm (Fig. 6C) and in the medial (SM) and lateral (SL) septal nuclei. In the SL, punctate fibers surrounding negative cell bodies are often observed (Fig. 5E). Most of the striatum and the hippocampus contain no VT-ir structures. Vasotocinergic innervation is present in the ventromedial capsular region around the nucleus robustus archistriatalis (RA; Fig. 7C–D). In the hypothalamus, ir fibers are widespread in many regions including the POA, the periventricular region, the POM (Figs. 1D–F, 7A–B), the hypothalamic ventromedial nucleus (VMN; Figs. 1F, 2A–B), and the dorsal hypothalamus. In the mesencephalon, vasotocinergic fibers are detected in the superficial layers of the optic tectum (TeO), in the dorsomedial portion of the nucleus intercollicularis (ICo; Fig. 7E–F), in the ventral area of Tsai, in the substantia grisea centralis (SGC), in the reticular formation, and near the nuclei of the III and IV nervi. Scattered fibers are observed in the raphe nuclei. No immunopositivity is found in the cerebellum. In the pons ir fibers are present in the locus coeruleus (LoC) and in the raphe nuclei. A relatively dense amount of VT-ir fibers also is found in the nucleus of the solitary tract and in the lateral medulla.

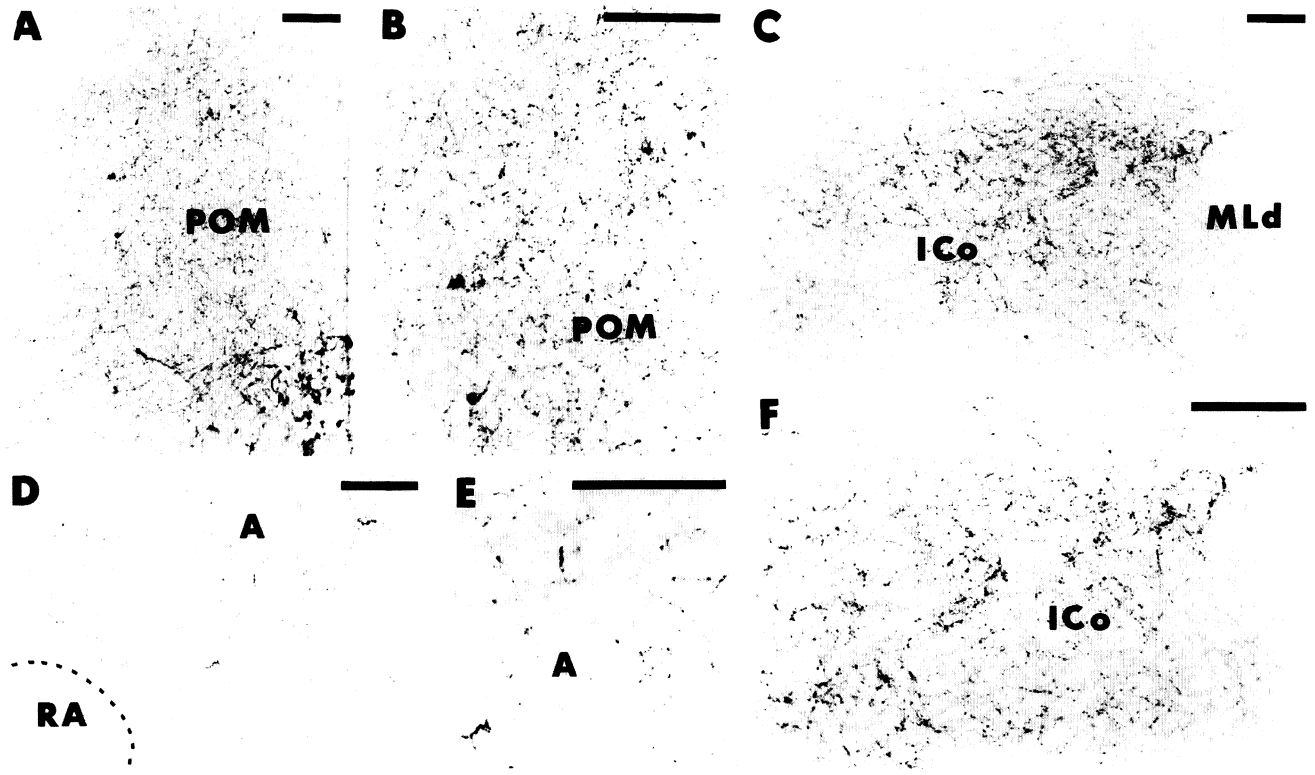

Fig. 7. Vasotocinergic innervation of the nucleus preopticus medialis (POM), ventromedial region of the archistriatum surrounding the nucleus robustus archistriatalis (RA), and the nucleus intercollicularis (ICo) nuclei. **A,B:** POM. **B:** Higher magnification view shows the presence of stained fibers and of some weakly stained cells. **D,E:** Sparse immunoreactive fibers surrounding the RA. **C,F:** Dorsomedial portion of the ICo. Note the absence of immunoreactivity in the adjacent nucleus mesencephalicus lateralis, pars dorsalis (MLd). For abbreviations, see list. Scale bars = 100 μm.

DISCUSSION

VT-ir system distribution

In adult, photostimulated male juncos, VT-ir cell bodies are located in three prosencephalic regions: the periventricular and lateral hypothalamic regions and a dorsal region that extends from the dorsal hypothalamus to the limbic region of the telencephalon. Immunoreactive fibers are widespread in several encephalic regions including diencephalon, telencephalon, mesencephalon, pons, and medulla. This distribution pattern resembles that described in other avian species, both nonoscine and oscine, and is most similar to that found in the canary and the zebra finch (Kiss et al., 1987; Voorhuis and De Kloet, 1992; Balthazart et al., 1996).

In galliforms, VT-ir cell bodies of the preoptic periventricular region are scattered along the third ventricle wall and clustered in at least three subdivisions (Viglietti-Panzica, 1986; Sánchez et al., 1991); in juncos, these cell bodies are less scattered along the ventricular wall and more concentrated in a single region. Moreover, we detected a similarity between galliforms and the junco in the lateral hypothalamic region only with respect to the distribution of supraoptic clusters and not to the localization of the SCN (Panzica, 1985; Norgren and Silver, 1990). The exact location of the SCN in galliforms is controversial. According to the stereotaxic atlas by Kuenzel and Masson (1988), the SCN can be divided into medial and lateral parts. The lateral part coincides with the nucleus of the supraoptic decussationis (Wedner et al., 1985). It receives direct optic fibers from the retina and contains melatonin binding sites (Wedner et al., 1985; Cozzi et al., 1993), but apparently no VT-ir cells. In contrast, only a few studies have identified optic fibers projecting to the medial portion of SCN (Bons, 1976; Csillag et al., 1995), which contains a well-recognizable cluster of VT-ir cells (Panzica, 1985; Norgren and Silver, 1990). The distribution of VT-ir cell bodies and of melatonin binding sites (Cassone et al., 1995) in the junco and in other oscine bird brains constitute one feature that suggests that the location of the oscine SCN corresponds to that of the lateral SCN in galliforms (Cassone and Moore, 1987).

The dorsal diencephalic group of ir elements has been identified with the BSTm described in both oscines (canary: Kiss et al., 1987; Voorhuis et al., 1988b; Voorhuis et al., 1990) and nonoscines (Aste et al., 1998). In galliforms, some medium and large ir neurons have been found in the more rostral part of the dorsal thalamus (Viglietti-Panzica, 1986; Jurkevich et al., 1997). These elements are absent in juncos and other oscine species (Kiss et al., 1987; Voorhuis and De Kloet, 1992).

The present data extend observations done on the zebra finch and suggest the need to reorganize the nomenclature of the oscine preoptic–anterior hypothalamic region. Balthazart et al. (1996) described the location of the POM and the PVN by using sections that were stained alternately for aromatase (the enzyme responsible for converting testosterone into estradiol and a good marker of the POM) and for VT (to identify the magnocellular elements

of the PVN). As illustrated in their Figure 7, the plane of tissue sectioning generally used in oscine differs markedly from that used to section galliform bird brains. This difference results in different relationships among neuroanatomical structures (Nissl-stained cell clusters, fiber tracts) that are normally used as landmarks to identify brain nuclei. Consistent with these observations, we have demonstrated that the junco paraventricular nucleus (identified as the major cell groups of VT-ir neurons of the periventricular region) is located ventrally to the so-called paraventricular magnocellular nucleus described in the canary atlas (Stokes et al., 1974). This last cluster of neurons, which was clearly visible in our Nissl-stain preparations, corresponds to the POM described in galliforms (Panzica et al., 1991, 1996) and in other oscine birds (Balthazart et al., 1996) in terms of shape and position. Preliminary results confirmed that the junco POM contains numerous aromatase-ir cell bodies (Plumari et al., 1998). The VT-ir fiber distribution of juncos is similar to that of the canary and the zebra finch.

Potential roles of the central VT system

The widespread distribution of VT-ir cell bodies and fibers in brain regions serving different roles suggests the involvement of this peptide in the control of multiple neurophysiological and behavioral functions.

The present observations extend those of previous investigations on canaries and zebra finches. In these species, as in juncos, VT-ir fibers have been identified in the capsular area surrounding the RA (Kiss et al., 1987; Voorhuis et al., 1991b; Voorhuis and De Kloet, 1992), a region that is specifically involved in the control of singing behavior (Nottebohm et al., 1976; Vicario, 1991; Vicario and Simpson, 1995). A role for VT in the control of this behavior in canaries is suggested by the fact that peripheral administration of a putative VT receptor antagonist to males altered their song duration and their probability to sing (Voorhuis et al., 1991a). It is of interest to note that canary RA VT-ir expression does not change seasonally or as a function of circulating gonadal steroid concentrations (Voorhuis et al., 1991b). Thus, seasonal and/or gonadal steroid-induced changes in singing behavior may not concur with alterations in RA VT expression. In adult canaries, RA contains putative VT receptors (Voorhuis et al., 1988a). Testosterone administration to females of this species induces singing (Leonard, 1939; Nottebohm, 1980) and increases the density of their RA VP binding sites (Voorhuis et al., 1988a). Thus, alterations not of VT expression but of the local density or activity of receptors for this peptide may mediate the behavioral and/or neuroanatomical effects of testosterone on RA. Another brain area that contains VT-ir fibers and terminallike structures (Kiss et al., 1987; Voorhuis et al., 1988b; De Kloet and Voorhuis, 1992; Viglietti-Panzica et al., 1997; present study) and participates in the control of vocalizations is the dorsomedial portion of the ICo (Newman, 1970; Brown, 1971; Phillips et al., 1972; De Lanerolle and Andrew, 1974; Armitage and Seller, 1981; Seller and Armitage, 1983). ICo contains androgen binding sites (zebra finch: Arnold et al., 1976; Japanese quail: Balthazart et al., 1992). VT-ir expression in this region is, however, apparently not affected by testosterone administration, and ICo VP binding sites have not been identified in canaries (Voorhuis et al., 1988a,b). The role of the ICo VT-ir system in vocal behavior control is, therefore, presently unknown.

VT-ir cell bodies and/or fibers are widely distributed in regions of the avian brain, including the POM, the BST, and the septum, that play important roles in the control of reproductive (Viglietti-Panzica, 1986; Kiss et al., 1987; Robinzon et al., 1988; Voorhuis et al., 1991b; Viglietti-Panzica et al., 1992, 1994; Jurkevich et al., 1996; Aste et al., 1998) and aggressive (Goodson, 1998a,b; Goodson and Adkins-Regan, 1999; Goodson et al., 1998) behaviors. These regions are well-established sites of gonadal steroid actions (for a recent review, see Panzica et al., 1997,) and their VT-ir system has frequently been associated with aromatase-ir neurons (Balthazart et al., 1997). In particular, the quail POM has been identified as a key region controlling male copulatory behavior (Panzica et al., 1996). An important role for central steroid-sensitive VP/VT pathways in the control of sexual behaviors has been identified in mammals (Södersten et al., 1986; Smock et al., 1992; for review, see De Vries, 1995) and in amphibians (for reviews, see Moore, 1992; Moore et al., 1994; Boyd, 1997).

Many of these studies suggest that VP or VT stimulates the expression of reproductive behaviors, although inhibitory effects of these peptides have also been demonstrated (e.g., inhibition of release call in *Rana pipiens*, of spontaneous locomotion in *Rana catesbiana*, and of lordosis in female rat: Diakow, 1978; Södersten et al., 1983; Moore, 1992). Little experimental work on this subject has been carried out in birds. Previous studies have shown that peripheral injection of VT to intact sexually mature pigeons or chickens increases the frequency of copulation (Kihlström and Danninge, 1972). More recently, data presented in abstract form have suggested that VT decreases motivational aspects of sexual learning in quail (Bernroider and Leutgeb, 1994). Recent studies have indicated that overt aggression is facilitated by central VT administration in the zebra finch (Goodson and Adkins-Regan, 1999) but is inhibited in two territorial species (*Spizella pusilla*: Goodson, 1998a; *Uraeginthus granatina*: Goodson, 1998b). In contrast, this treatment to female Zebra Finch can elicit singing (Maney et al., 1997). Finally, either periphally or centrally injected VT inhibits many aspects of sexual behavior in male quail (Castagna et al., 1998). Specifically, appetitive and consummatory components of sexual behavior were dose-dependently depressed by VT injected into the third ventricle in this species. Effects in the opposite direction were observed after injection of a VT antagonist, indicating that in physiological conditions endogenous VT tonically inhibits sexual behaviors.

In conclusion, the results of the present and previous studies strongly suggest the involvement of VT in the control of gonadal hormone-mediated reproductive behaviors in avian species.

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